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Comparative Ethanol Productivities of Two Different Recombinant Fermenting Strains on Source-Separated Organic Waste

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ABSTRACT
Production of biofuel such as ethanol from lignocellulosic biomass is a beneficial way to meet sustainability and energy security in the future. The main challenge in bioethanol conversion is the high cost of processing, in which enzymatic hydrolysis and fermentation are the major steps. Among the strategies to lower processing costs are utilizing both glucose and xylose sugars present in biomass for conversion. An approach featuring enzymatic hydrolysis and fermentation steps, identified as separate hydrolysis and fermentation (SHF) was used in this work. Proposed solution is to use "pre-processing" technologies, including the thermal screw press (TSP) and cellulose-organic-solvent based lignocellulose fractionation (COSLIF) pretreatments. Such treatments were conducted on a widely available feedstock such as source separated organic waste (SSO) to liberate all sugars to be used in the fermentation process. Enzymatic hydrolysis was featured with addition of commercial available enzyme, Accellerase 1500, to mediate enzymatic hydrolysis process. On average, the sugar yield from the TSP and COSLIF pretreatments followed by enzymatic hydrolysis was remarkable at 90%. In this work, evaluation of the SSO hydrolysate obtained from COSLIF and enzymatic hydrolysis pretreaments on ethanol yields was compared by fermentation results with two different recombinant strains: Zymomonas mobilis 8b and Saccharomyces cerevisiae DA2416. At 48 hours of fermentation, ethanol yield was equivalent to 0.48g of ethanol produced per gram of SSO biomass by Z.mobilis 8b and 0.50g of ethanol produced per gram of SSO biomass by S. cerevisiae DA2416. This study provides important insights for investigation of the source-separated organic (SSO) waste on ethanol production by different strains and becomes a useful tool to facilitate future process optimization for pilot scale facilities.

Keywords – enzyme, ethanol, lignocellulose, organic waste

I. INTRODUCTION

Ethanol production from lignocellulosic biomass has a potential to be a viable replacement or supplement for fossil fuel, but the current cost of conversion is a major bottleneck for commercial application [1]. The price for ethanol remains as high as $2.75 per gallon motivating further research [2]. By contrast the average price for regular, unleaded gasoline in the USA is currently hovering around $3.9 per gallon with expectation for it to rise even more [3]. It became apparent that in efforts to reduce the production costs of ethanol, improvements in several areas of biofuel production including feedstock, price design and enzymes are required. At the present time, there are at least two methods of ethanol production from lignocellulose that are in advanced phases of development: enzymatic hydrolysis and biomass fermentation. Neither process generates toxic emissions while producing the end product, which is ethanol. The technology is relatively new and exists in pilot configurations where testing is ongoing. While today ethanol is mostly produced from starch contained in grains such as corn, sugarcane and grain sorghum, it can also be produced from cellulose which is mainly present in non-food products. Currently, lignocellulosic feedstock is the most abundant biomass, which has attracted considerable attention and is often a major or the sole component of different waste streams from various industries including agriculture, forestry and municipalities' wastes [4].

Today's bioethanol technology has offered sustainable approaches to the problem with municipal solid waste (MSW) by focusing on utilization of organic fraction of solid waste and agriculture residue in order to reduce wastes and avoid conflicts between human food and industrial use of crops. Organic fraction of solid waste has given a new perspective to the industry by defining an innovative system for converting trash into bioethanol reducing the amount of waste piling up in landfills, while displacing a large fraction of the fossil fuels to power vehicles. Biomass such as processed source separated organic (SSO) waste is particularly attractive in one context

since it is widely available at a negative cost and has many other environmental benefits. It provides a good alternative fuel in terms of green-house gas emissions, reduction of farmland's depletion, and diminutive of generated waste.

Ethanol yield and productivity are the key parameters in the production of biofuel from biomass and wastes. The fermentation of xylose-to-ethanol is important in biomass-to-ethanol process since it can increase ethanol yield up to 50% [5]. Several strains have been engineered to ferment xylose to ethanol as per [6-8]. Among them are Zymomonas mobilis, Saccharamyces cerevisiae, and Pitchia stipulus. The first two abovementioned strains met the selection criteria which were based on several fermentation characteristics considered to be essential for biomass-to-ethanol conversion [9-10].

The purpose of this study was a comparison of the growth and fermentation performances of pretreated source-separated organic (SSO) waste on ethanol productivities of two glucose/xylose utilizing recombinant strains: Zymomonas mobilis 8b and Saccharomyces cerevisiae DA2416.

The feasibility of the SSO as a potential feedstock for ethanol production has been demonstrated in [11-16]. Before pre-treatment, a compositional characterization of pre-processed SSO samples collected at the City of Toronto, Ontario, Canada for a ten-month period was carried out as in [13].

II. MATERIALS AND METHODS

The SSO waste samples intended in this research were pre-processed mechanically under high temperature and pressure by the thermal screw press (TSP) and then used as a substrate for all enzymatic hydrolysis and fermentation processes. Moreover, the SSO waste samples were made as a heterogeneous substrate of demolished construction waste blended with 20% of woodchips plus 20% organic green bin waste and pre-processed accordingly [17]. Prior to testing the SSO waste was oven dried at 45°C-50°C for 48 hours.

The next step encompassed lignocellulosic fractionation by cellulose solvent (phosphoric acid) and organic-solvent (ethanol). Five grams of dry lignocelluloses was placed in a 250 mL centrifuge bottle and then mixed with 40 mL of 85% concentrated phosphoric acid using a glass rod. The solid/ liquid slurry was placed in a benchtop shaking incubator at 150rpm and 50 ℃ ± 0.2℃ for two hours. One hundred mL of ethanol was then added and mixed well. After centrifugation at 7000 rpm at room temperature for 15 minutes, the supernatant was decanted. The solid pellet was then re-suspended by 200 mL of ethanol and centrifuged. The supernatant again was decanted. Next, the solid pellet was re-suspended by 200 mL of distilled water and centrifuge two times and stored in a freezer for a short period of time.

Enzymatic hydrolysis experiments were carried out with the addition of a commercially available enzyme, Accellerase 1500. After thawing, the treated solid pellet containing amorphous cellulose was neutralized to pH 4.8-5.0 by NH_4OH, a source of nitrogen. The SSO samples were then brought to 50℃ before adding 30 FPU/ g glucan of Accelerase 1500. Both the pH value and temperature described were the optimum conditions for the Accelerase 1500 enzyme to mediate hydrolysis and release fermentable sugars as much as possible. The hydrolysis experiment was conducted in the benchtop shaking incubator. The incubator was set at 250 rpm to keep solids in constant suspension with the temperature of 50°C for 72 hours. Samples were taken for sugar content at specified times: 0, 12, 24, 48 and 72 hours to measure sugar content. The relevant composition of the SSO was 33% (w/v) glucose, 19% (w/v) xylose and 3% (w/v) acetic acid.

Following enzymatic hydrolysis, batch soluble sugar fermentation was carried out to evaluate ethanol yields by performance of two different recombinant strains: Z. mobilis 8b and S. cerevisiae DA2416. Soluble sugars batch fermentation was performed in 250 mL serum bottles with 100 mL working volume and purged before being autoclaved. Temperature was maintained at 30^{o}C and pH was controlled at 6.0 by 1M potassium hydroxide (KOH) as suggested by previous studies [18]. Compositional analysis of the samples in duplicates for ethanol concentrations was carried out at 0, 12, 24 and 48 hours by high performance liquid chromatography (HPLC). The metabolic ethanol yield (Ym) was calculated as a mass of ethanol produced per mass of sugar consumed. The process ethanol yield (Yp) was obtained by dividing the ethanol concentration by total sugar concentration in the feed medium. The volumetric ethanol productivity was derived by ratio of ethanol concentration and time taken to complete fermentation (48 hours).

III. RESULTS AND DISCUSSION

Due to its potential for industrial application, the SSO waste was chosen as the substrate to evaluate the values on sugar and ethanol yields by fermentation using Z. mobilis 8b and S. cerevisiae DA2416 strains. Detailed quantitative assessment on the composition of SSO waste was completed prior to this study [13], and the results are presented in Table 1.

Table 1: Compositional analysis of (SSO) sample

Parameters	Average Value
A. Physical Properties	
Biomass as received	
pH	5.2 @ 25°C
Total Solids (TS)	44.33%
Moisture content	55.66%
Volatile organic compound (VOC) per dry mass	13.66%
Ash per dry mass	5.14%
Oven-dried and homogenized biomass	
pH	5.5 @ 25°C
Moisture content	6.60%
TS	93.40%
VOC	86.33%(TS)
Ash	13.60% (TS)
B. Sugars and Lignin (per oven-dried and homogenized biomass)	
Glucose	31%
Xylose	19%
Other sugars	9%
Total sugars	59%
Total Lignin	23%
C. Others	
Total Kjehldahl Nitrogen (TKN)	9198 µg/g
Extractives	7%
Calorific value	16961.6 kj/kg

As seen in Table 1, approximately, more than half of the original sample is composed of moisture. Essential polymeric sugars in an oven dried SSO samples included: 33% glucose, 19% xylose, and about 9% of other sugars and 23% of lignin. These homogeneous samples with pH at 5.2-5.5 had around 20% of the food waste and a 20% of wood chips (Douglas fir type).

Enzymatic hydrolysis and fermentation experiments were next in the line to be conducted in sequence in the chosen SHF approach. The whole process usually takes five days to complete. The SSO samples pretreated by concentrated phosphoric acid (85% w/w) and ethanol (95% v/v) were hydrolyzed fast and glucan digestibility were found to be 72% after 24 hours and 90% after 72 hours. The high glucan digestibility seen in Fig. 1 was achieved for the COSLIF-pretreated SSO with addition of 30 FPU/ g glucan of Accelerase 1500.

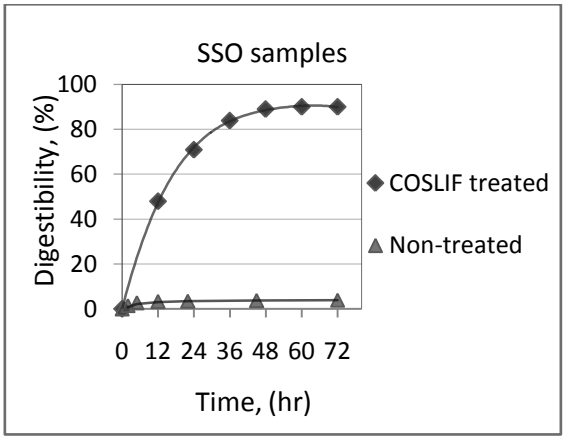

Fig. 1: Glucan digestibility profiles for COSLIF treated and untreated SSO samples

This result was mainly attributed to drastic changes in surface morphology of intact and COSLIF-pretreated SSO samples. The intact SSO has obviously maintained its tight micro-fibril structure, while a COSLIF-pretreated sample evidenced homogeneous biomass as seen in our previous work [12]. The enzymatic glucose digestibility for pre-treated COSLIF samples was calculated as described in [19]. We hypothesized that almost all lignin have been removed from SSO waste sample during COSLIF and enzymatic hydrolysis phases. But it would be impractical to completely wash cellulose solvents out, as it requires a large amount of water. Negative effects of residual lignin on enzymatic hydrolysis may contribute to 1) enzyme adsorption by lignin, 2) obstruction of lignin on the surface of cellulose to that point when enzyme are not able to access cellulose [2], [20].

In a separate series of experimental evaluation, enzymatic hydrolysate obtained from COSLIF pretreament by batch culture fermentation with Z. mobilis 8b strain, was compared with S. cerevisiae DA2416. Fig. 2 shows the glucose and xylose consumption trajectoires for fermentation of the SSO pretreated samples.

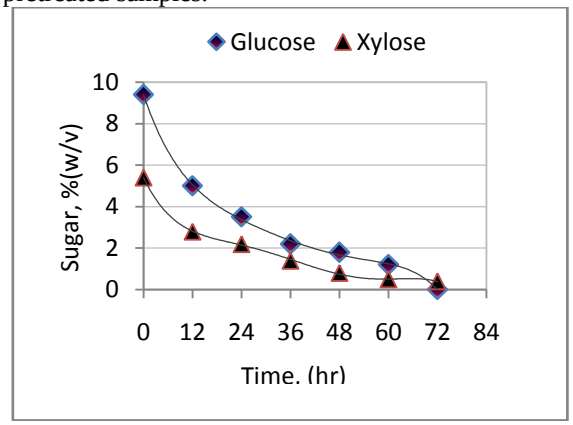

Fig. 2: Sugar consumption profiles of the SSO pretreated hydrolysates during fermentation phase

Whereas both strains exhibited almost the same value of ethanol yields based on sugar consumed (0.48g/g and 0.5g/g) as seen in Figure 3, the process yield on the total initial sugar concentration was 0.48g/g for Z. mobilis 8b and 0.49g/g for S. cerevisiae DA2416 (See Table 2). After 72 hours, glucose is completed decomposed, while a small amount of xylose remains. It has been documented that the main substrate for Z. mobilis 8b is glucose, while S. cerevisiae DA2416 decompose both glucose and xylose. Therefore the production of ethanol is higher for the S. cerevisiae DA2416 strain after glucose is used up. The significantly better performance of S. cerevisiae DA2416 compared to Z. mobilis 8b suggests a possible role of inhibitors other than acetic acid on bacterial growth in fermentation phase, for example phenolic compounds from lignin and etc. It is both well known and documented [21-23] that ethanol is an inhibitor to xylose utilization by Z. mobilis 8b with ethanol concentration of 5.5%-6% (w/v) causing complete deceleration of the process.

In further fermentation assays with the Z. mobilis 8b strain, after 48 hours, 100% of glucose and 40% of xylose were consumed. On the other hand, in the enzymatic hydrolysate with S. cerevisiae DA2416, fermentation advanced more rapidly, with 100% glucose and 60% xylose consumed after the same period of time. The growth and fermentation parameters of this work are summarized in Table 2.

Table 2: Growth and fermentation parameters

Strains	**Z.mobilis 8b**	**S.cerevisiae DA2416**
Total amount of sugar, % (w/v)	14.8	14.8
Glucose, % (w/v)	9.5	9.5
Xylose, % (w/v)	5.3	5.3
Acetic acid, % (w/v)	1.0	1.0
Process yield, g/g	0.48	0.49
Metabolic yield, g/g	0.48	0.50
[1]Productivity, g/L·h	0.88	0.92
Ethanol yield, g/L	140	152

[1]Productivity data was based on a fermentation time of 48 hours
Process yield was based on available sugars
Metabolic yield was based on sugar utilized

The fermentation was complete at 48 hours (Fig. 3) with a final ethanol concentration of 4.5% (w/v) representing a volumetric productivity of 0.92g/(L•h) and ethanol yield of 0.5g/g or 96% theoretical maximum conversion efficiency for performance with S. cerevisiae DA2416. The final ethanol concentration 3.5% (w/v) represented a volumetric productivity of 0.88g/(L•h) and an ethanol yield of 0.48g/g or 94% theoretical maximum conversion efficiency for performance with Z. mobilis 8b.

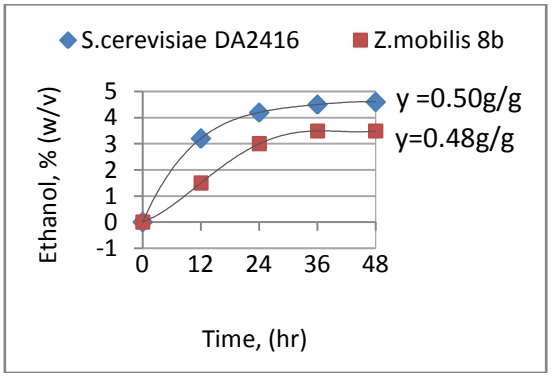

Fig. 3: Comparative fermentation performance of both strains for ethanol production in time range of 48 hours

In summary, low bacterial activity in fermentation of SSO hydrolysate by Z. mobilis 8b may be attributed to many other factors, including: longer lag phase - an adaptation time for growth condition of chosen strain, low growth rate on SSO hydrolysate and lack of micronutrients such as nitrogen, phosphorus.

IV. CONCLUSIONS

The SSO waste samples utilized in this research were pre-processed by the thermal screw press (TSP) and further used as substrates for all enzymatic hydrolysis and fermentation processes.

COSLIF pretreatments were applied for cellulose extraction from processed source separated organic waste. Results indicated that the percent glucan conversion was considerable for COSLIF pretreated samples compared to untreated samples. This study demonstrated and affirmed that S. cerevisiae DA2416 outperformed Z. mobilis 8b on ethanol yields during fermentation process. However, a more comprehensive investigation on lignocellulosic usage with different enzymes and recombinant fermenting strains would be advantageous in biofuel field.

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